



Biotinylation of Protein for Immobilization onto Streptavidin Biosensors

OVERVIEW

The interaction between streptavidin and biotin is widely used as a system for the rapid, stable and irreversible non-covalent binding of biological molecules. The Octet® platform's Streptavidin biosensors have been developed for the immobilization of biotinylated ligands for both quantitation and kinetic applications. The first protein immobilized onto the Streptavidin biosensors must be biotinylated prior to assaying on the Octet system.

This technical note supersedes ForteBio Technical Notes 6 (*Biotinylation of Protein for Immobilization onto Streptavidin K Biosensors*) and 12 (*Biotinylation of Very Small Quantities of Protein for Immobilization onto Streptavidin Biosensors*). It provides guidelines for biotinylating a protein of interest utilizing Pierce-ThermoFisher (piercenet.com) biotinylation reagents for kinetic and quantitation assays with Streptavidin biosensors on the Octet platform.

MATERIALS REQUIRED

- EZ-Link NHS-PEG₄-Biotin (Thermo, part no. 21329) prepared as a 1mM solution in water
 - Can also use the NHS-PEG₁₂-Biotin (Thermo, part no. 21312) or Sulfo-NHS-LC-LC-Biotin (Thermo, part no. 21338)
- Distilled water
- 1X PBS
- Zeba desalting spin columns for buffer exchange
 - For protein samples of 30–130 µL, use 0.5 mL columns (Thermo, part no. 89882)
 - For protein samples of 200–700 µL, use the 2 mL columns (Thermo, part no. 89889)

- For protein samples of 600–2000 µL, use the 5 mL columns (Thermo, part no. 89891)
- Protein to be biotinylated (requires at least 50 µg total at a concentration of at least 100 µg/mL)
- (Optional) Slide-A-Lyzer (Thermo, part no. 66370)

PROTEIN SAMPLE PREPARATION

- Ensure that the protein is carrier-protein free (for antibodies containing carrier protein, see ForteBio Technical Note 11.)
- Ensure that the protein to be biotinylated is not in a buffer containing primary amines (*i.e.*, Tris or glycine). If protein is in a buffer containing primary amines, exchange into PBS either by dialysis or desalting spin columns. The protein concentration is recommended to be at least 1 mg/mL for this process.

Protein Sample Preparation by Dialysis (recommended for samples >0.5mL)

- 1 Dialyze each sample 1:1000 in 1X PBS using a Slide-A-Lyzer.
- 2 Allow the protein and Slide-A-Lyzer to stir gently in PBS for a minimum of 3 hours before changing the PBS.
- 3 Change PBS at least 4 times before extracting the protein.

Protein Sample Preparation by Desalting Column (recommended for samples <0.5mL)

- 1 Follow the manufacturer's instructions for using the desalting columns for buffer exchange.

$$\mu\text{L } 1 \text{ mM biotin reagent} = \frac{\text{Protein Conc (mg/mL)}}{\text{MW Protein (k}_{\text{Da}})} \times \text{MCR} \times \text{volume Protein } (\mu\text{L})$$

Example 1: 1 mL solution of IgG (150 k_{Da}) at 1 mg/mL with an MCR of 1:

$$\mu\text{L } 1 \text{ mM biotin reagent} = (1/150) \times 1 \times 1000 = 6.67 \mu\text{L}$$

Example 2: 0.5 mL solution of IgG (150 k_{Da}) at 0.5 mg/mL with an MCR of 1:

$$\mu\text{L } 1 \text{ mM biotin reagent} = (0.5/150) \times 1 \times 500 = 1.67 \mu\text{L}$$

Example 3: 0.5 mL solution of IgG (150 k_{Da}) at 0.5 mg/mL with an MCR of 5:

$$\mu\text{L } 1 \text{ mM biotin reagent} = (0.5/150) \times 5 \times 500 = 8.33 \mu\text{L}$$

FIGURE 1: Equation for calculating the required volume of 1 mM biotin reagent.

BIOTIN CALCULATION AND PREPARATION

The amount of 1 mM biotin reagent required depends on the molar coupling ratio (MCR) of the biotin reagent to protein. In general, it is recommended to use a 1:1 ratio (1 biotin for every protein molecule). If the protein concentration is less than 500 µg/mL or if the extent of biotinylation at an MCR of 1:1 is found to be insufficient, a 3:1 or 5:1 ratio is recommended.

- 1 Based on the selected MCR, calculate the volume of 10 mM biotin reagent needed. A sample calculation is shown in Figure 1.
- 2 Prepare a 1 mM biotin reagent solution.
 - a If using NHS-PEG₄-Biotin, pierce the foil top and add 170 µL of distilled water to the tube to create a 20 mM stock solution. Add 50 µL of the 20 mM biotin stock solution to 950 µL of distilled water to create a 1 mM solution of NHS-PEG₄-Biotin.
 - b For the NHS-PEG₁₂-Biotin, the product insert instructs on how to create a 250 mM stock solution; add 4 µL of this stock solution to 996 µL water to create a 1 mM solution.
 - c For the sulfo-NHS-LC-LC-Biotin, the product insert instructs on how to create a 10 mM stock solution; add 100 µL of this stock solution to 900 µL distilled water to create a 1 mM solution.
- 3 To each sample, add the appropriate volume (µL) of Biotin reagent as calculated in Step 1.
- 4 Mix immediately.
- 5 Incubate 30 minutes at room temperature.
- 6 Stop the reaction by removing the excess biotin reagent using the desalting column (follow manufacturer's product insert).